BIOACTIVE DOSE OF DIOXIN-LIKE COMPOUNDS IN BLOOD SAMPLES OF A POPULATION EXPOSED TO CHEMICAL WASTE OF A PCP FACTORY IN TAIWAN

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Abstract

Pentachlorophenol (PCP) and chemical waste produced by a factory in southwestern Taiwan continued to contaminate the surrounding area even after the plant closed. Sera PCDD/PCDF levels of residents in the contaminated area have been found to be higher than those of non-contaminated area. These residents may have higher AhR ligand levels in their blood, which would probably raise the AhR activity abnormally and might lead to related health effects. The standard clean-up method for Calux (Chemically Activated Luciferine gene eXpression) bioassay focusing on PCDDs, PCDFs and PCBs, trying to fulfill the WHO TEF values[18], would eliminate PAH[19], among other known AhR ligands. Although not a persistent chemical, the presence of AhR is constant the environment. Depletion of PAH from samples for bioassy may lead to underestimation of the AhR ligand levels. In this work we used an alternative clean-up method conserving PAH, in order to maximize the estimation of the biological dose of AhR activators in blood samples of the above mentioned population. As expected, the cleaned-up products of these columns had higher Calux response. Good correlation was observed for Calux results expressed either as RLA, RLA/g fat or RLA/g blood. Till the moment, we found no correlations between RLA and BMI, gender or body lipid content. Neither any correlation was observed between RLA, RLA/g fat or RLA/g blood and hepatic function parameters (ALT, AST), cholesterol level or percentage of fat in blood. However, the sample size (n=36) is still low to draw final conclusions.

Introduction

The toxic and biological effects of TCDD and dioxin-like compounds are mediated by aryl hydrocabon receptor (AhR)[1]. Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polychlorinated naphthalenes (PCNs), hexachlorobenzene, and derivatives of these compounds can also activate AhR[2]. AhR-dependent toxicity appears to be primarily driven by abnormal and persistent activation of AhR-dependent gene expression in target cells[3]. Natural and endogenous ligands of AhR -including flavonoids, carotenoids, tryptophan, and arachidonic acid metabolites- have relatively weak affinity compared to TCDD and are rapidly degraded[4]. Human exposure to PCDD/Fs and PCBs is associated with serious consequences to health including developmental[5,6], neurological[7], immunological[8], reproductive[9-12], and carcinogenic effects[13,14].

Pentachlorophenol (PCP) and chemical waste produced by a factory in southwestern Taiwan continued to contaminate the surrounding area even after the plant closed. Sera PCDD/PCDF levels of residents in the contaminated area have been found to be higher than those of non-contaminated area. These residents may have higher AhR ligand levels in their blood, which would probably raise the AhR activity abnormally and might lead to related health effects.

Chemically Activated Luciferine gene eXpression (CALUX) is a valid method for the screening of all compounds with affinity to AhR, including PCDD/Fs and dioxin-like PCBs in biological and environmental samples, e.g. serum[15], plasma[16] or milk[17].

The standard clean-up method for Calux bioassay focusing on PCDDs, PCDFs and PCBs, trying to fulfill the WHO TEF values[18], would eliminate PAH[19], among other known AhR ligands. Although not a persistent

chemical, the presence of AhR is constant the environment. Depletion of PAH from samples for bioassy may lead to underestimation of the AhR ligand levels. In this work we present the results of an alternative clean-up method conserving PAH, in order to maximize the estimation of the biological dose of AhR activators in blood samples of the above mentioned population.

Materials and Methods

Bio-analyses were performed at Hiyoshi Corporation, Japan. Lipid fraction of 10 mL of whole blood with heparin was extracted with acetone and n-hexane, cleaned up using a celite-sodium sulphate column, dried with nitrogen evaporator and transferred to different silica-gel columns in hexane.

Silica-gel column without acid silica gel
A pooled blood sample was used to test the clean-up effects of the different columns.

	Column A (standard procedure), coupled with XCARB column	Column B	Column C
Composition of the silica gel columns	Sodium sulfate 45% sulphuric acid silica gel Silver nitrate silica gel Silica gel KOH silica gel Silica gel 45% sulphuric acid silica gel Sodium sulfate Glass wool	Sodium sulfate Silver nitrate silica gel Silica gel KOH silica gel Silica gel Sodium sulfate Glass wool	Sodium sulfate Silica gel Sodium sulfate Glass wool

Columns B and C are versions of column A without the 45% sulphuric acid silica gel layers, which would retain PAH. The cleaned-up products of these columns are expected to have a higher Calux response.

Blood samples from contaminated area residents
Thirty-six blood samples from the exposed population have been processed with the column B as described. The resulting fractions were evaporated and used to treat H1L6.1 cell line for 20 hours. Calux results was expressed as relative luciferase activity (RLA), RLA per gram fat (RLA/g fat), and RLA per gram blood (RLA/g blood). Luciferase activity of 1 nM TCDD has RLA=1. Statistical analyses were performed with SPSS 15.0.

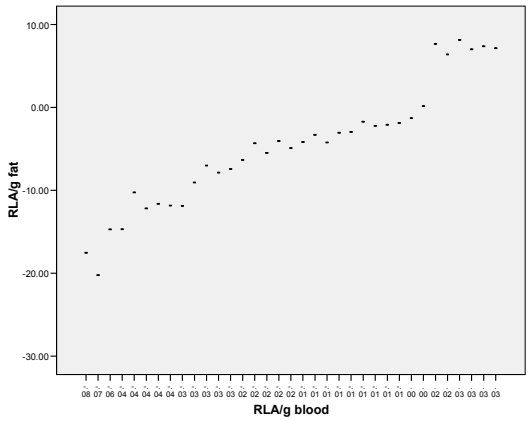
Results and Discussion

Calux results using silica-gel columns with or without the 45% sulphuric acid silica gel layers

Silica-gel column used	Column A (standard method)		Column B	Column C
	DL-PCB	PCDD/F		
RLA	0.1774	0.4127	0.5843	0.3515
Blood sample amount	8 ml	8 ml	1ml	1ml

Correlations among different Calux expressions
Good correlation was observed for Calux results expressed either as RLA, RLA/g fat or RLA/g blood. Further analyses can use either of the expressions.

Correlations between	Pearson correlation
RLA and RLA/g blood	1
RLA and RLA/g fat	0.984
RLA/g blood and RLA/g fat	0.985



Calux results and health parameters of the contaminated area residents

While performing the lipid extraction of these samples, different colors of lipids were observed in extractions from different subjects, ranging from strong orange to pale yellow. Some samples still had color after passing the column B, others showed cytotoxicity or changed the color of the culture media in later steps.

We found no correlations between RLA and BMI, gender or body lipid content. No correlation was observed between RLA, RLA/g fat or RLA/g blood and hepatic function parameters (ALT, AST), cholesterol level or percentage of fat in blood. However, the sample size is still low to draw final conclusions.

Further studies of this project include AhR expression, levels of cancer biomarkers, and level of DNA damage. A questionnaire concerning nutritional habits and general lifestyle will also supplement the biological data. At the end of this work, we should obtain concrete information about the health impact produced by the dioxin and dioxin-like compounds in the area; we would have direct evidences on the relationship among the level of bioactive dioxin-like compounds, AhR activity and related clinical parameters in the exposed population.

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